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Population Pharmacokinetics of Lumefantrine in Pregnant Women Treated with Artemether-Lumefantrine for Uncomplicated *Plasmodium falciparum* Malaria▽

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Artemether-lumefantrine has become one of the most widely used antimalarial drugs in the world. The objective of this study was to determine the population pharmacokinetic properties of lumefantrine in pregnant women with uncomplicated multidrug-resistant *Plasmodium falciparum* malaria on the northwestern border of Thailand. Burmese and Karen women ($n = 103$) with *P. falciparum* malaria and in the second and third trimesters of pregnancy were treated with artemether-lumefantrine (80/480 mg) twice daily for 3 days. All patients provided five capillary plasma samples for drug quantification, and the collection times were randomly distributed over 14 days. The concentration-time profiles of lumefantrine were assessed by nonlinear mixed-effects modeling. The treatment failure rate (PCR-confirmed recrudescent infections at delivery) was high; 16.5% (95% confidence interval, 9.9 to 25.1). The population pharmacokinetics of lumefantrine were described well by a two-compartment open model with first-order absorption and elimination. The final model included interindividual variability in all pharmacokinetic parameters and a linear covariate relationship between the estimated gestational age and the central volume of distribution. A high proportion of all women (40%, 41/103) had day 7 capillary plasma concentrations of <355 ng/ml (which corresponds to approximately <280 ng/ml in venous plasma), a threshold previously associated with an increased risk of therapeutic failure in nonpregnant patients in this area. Predictive modeling suggests that a twice-daily regimen given for 5 days would be preferable in later pregnancy. In conclusion, altered pharmacokinetic properties of lumefantrine contribute to the high rates of failure of artemether-lumefantrine treatment in later pregnancy. Dose optimization is urgently needed.

Pregnancy has considerable effects on the pharmacokinetic properties of many of the drugs used to treat uncomplicated *Plasmodium falciparum* malaria. Whereas blood chloroquine and quinine concentrations are relatively unaffected (1, 25), artesunate, artemether, dihydroartemisinin, sulfadoxine, atovaquone, proguanil, and cycloguanil concentrations are all reduced in later pregnancy (14, 30–33). The reductions are often substantial, and as a result, antimalarial cure rates in pregnancy for any given antimalarial drug tend to be lower (24, 34). Unfortunately, pregnant women are especially vulnerable to malaria and the fetus is adversely affected.

The fixed combination of artemether and lumefantrine is the result of research undertaken by Chinese scientists and has become the most widely used coformulated artemisinin-based combination therapy (ACT). Artemether-lumefantrine has proved effective (cure rates, >97%) and safe in adults and children in trials conducted throughout the areas of the world affected by malaria (2, 13, 15, 20, 23, 35, 43, 44, 59). There is a reluctance to prescribe new drugs to pregnant women, and there have been concerns over the safety of artemisinin derivatives in early pregnancy. The current assessment of the benefits compared with the potential risks suggests that the artemisinin derivatives should be used to treat uncomplicated *P. falciparum* malaria in the second and third trimesters, as a result of documented experience with the treatment of more than 1,500 pregnant women (9, 40, 56). Evaluation of the new fixed ACTs is lagging behind these recommendations (53).

A recent preliminary study with 13 pregnant women with frequent venous plasma sampling for pharmacokinetic analysis showed that the levels of artemether, dihydroartemisinin, and lumefantrine exposure were lower in this group than in nonpregnant adult patients in the same area (32). The maximum concentration (C_{max}) of dihydroartemisinin (the principal metabolite of artemether) and total drug exposure (the area under the concentration-time curve from time zero to 8 h [AUC_{0-8}]) were 20% and 40% lower, respectively, in pregnant patients than in nonpregnant patients. The level of exposure to lumefantrine after the last dose (the AUC from 60 h to infinity [$AUC_{60-\infty}$]) was 6% (90% confidence interval [CI], 5 to 12%) lower in pregnant patients than in nonpregnant patients, but

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because elimination was more rapid, later exposure (AUC from 120 h to infinity [$AUC_{120-\infty}$]) was 49% (95% CI, 28 to 91%) lower in pregnant patients than in nonpregnant patients (32). The total exposure to lumefantrine has previously been shown to be a good predictor of outcome in the same population (55), although it is believed that the later concentrations in the third and subsequent parasite life cycles (>4 days) may be the main determinant (52). The day 7 concentrations of lumefantrine correlate well with total drug AUCs, and this measurement has therefore been used as a measure of drug exposure in clinical studies of artemether-lumefantrine (55). Indeed, there are reasons to believe that this may be a better determinant of the therapeutic response than the total AUC for many antimalarial drugs used today (54). Venous plasma lumefantrine concentrations of 175 ng/ml (42) and 280 ng/ml (11, 12) at day 7 have been suggested to be cutoffs below which there is a 25% and a 50% risk of therapeutic failure, respectively, in this area. A plasma lumefantrine concentration of >500 ng/ml on day 7 is associated with a treatment success rate of >90% (11, 55). A high proportion (38%) of the pregnant patients in the preliminary pharmacokinetic study described previously had day 7 lumefantrine concentrations below 280 ng/ml, suggesting that lower concentrations on day 7 may result in lower cure rates in this vulnerable group (32).

The main objective of the study described here was to investigate the pharmacokinetic properties of lumefantrine by use of a population-based modeling approach in pregnant women treated with the standard dose of artemether-lumefantrine.

MATERIALS AND METHODS

Study site and ethical approval. This study was conducted in an area along the northwestern border of Thailand with a low rate of seasonal malaria transmission. This is an area where multidrug-resistant *P. falciparum* is prevalent. The study was carried out at the weekly antenatal clinics run by the Shoklo Malaria Research Unit (SMRU). This pharmacokinetic study was nested into a larger comparative study of the safety and efficacy of artemether-lumefantrine versus those of artesunate monotherapy in pregnancy that has been published in full elsewhere (34). Approval for the study was granted by three independent bodies: the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; the Oxford Tropical Research Ethic Committee; and the Secretariat Committee on Research Involving Human Subjects of the World Health Organization. The trial was registered on 7 June 2005 (trial ISRCTN86353884).

Study design. Patients were enrolled through a system of weekly screening, in which at each antenatal clinic visit, women have a blood smear taken to detect malaria. Uncomplicated *P. falciparum* malaria was defined as a slide-positive infection with levels of asexual parasitemia between 6 parasites per 500 leukocytes and 40 parasites per 1,000 erythrocytes in the absence of signs of severe malaria. All parasitemic episodes during pregnancy were treated. This regular screening program has been shown to reduce morbidity and prevent mortality (41). Women positive for *P. falciparum* had blood spots taken for genotyping by PCR so that treatment efficacy could be assessed. All women were encouraged to deliver under supervision in the SMRU facilities. Complicated deliveries requiring delivery by cesarean section were referred to Mae Sot Hospital. Patients with known chronic disease (cardiac, renal, or hepatic disease or hemoglobinopathy), an inability to follow the antenatal clinic consultation, a history of alcohol or narcotic abuse, imminent delivery, signs of severe malaria, an inability to tolerate oral treatment, or allergy to artemisinin derivatives or lumefantrine were excluded from the study. Witnessed written consent or a thumbprint was obtained from every patient participating in the study. If consent was forthcoming, a full medical history and examination (including obstetric evaluation) were carried out by a physician and the results were recorded, as described previously (34).

Drug regimen. The pregnant women enrolled in this study received artemether-lumefantrine (20/120 mg artemether/lumefantrine tablets; Novartis, Basel, Switzerland) as four tablets twice a day for 3 days with 200 ml to 250 ml of chocolate milk containing approximately 6 g to 7 g of fat with each dose. The relative bioavailability of lumefantrine in healthy volunteers has been demonstrated to increase more than fivefold when it is administered with a meal rich in fat (>1.2 g) (4). The protocol for supervised drug treatment was 0, 8, 24, 36, 48, and 60 h. The exact dosing times were recorded and were used in the modeling.

Blood samples. Patients were admitted to the SMRU facilities in Mae La Camp, and a baseline blood sample was taken before any drug treatment was given. Capillary blood samples (approximately 200 μl to 300 μl) were taken from a finger prick and placed in hematocrit heparinized tubes on four occasions selected at random from the following time windows: 0 to 72, 72 to 96, 96 to 144, and 144 to 336 h after initial drug administration. The exact sample times were recorded and were used in the modeling. The blood samples were centrifuged at 2,000 × *g* for 10 min, and the plasma was stored in cryotubes and placed at −30°C. All samples were transferred within 5 days to the Mae Sot Laboratory, where they were stored at −80°C.

Drug analyses. Lumefantrine concentrations were determined by solid-phase extraction and liquid chromatography (LC) with UV detection, as described previously (3). The LC system was a LaChrom Elite system consisting of an L2130 LC pump, an L2200 injector, an L2300 column oven set at 25°C, and an L2400 UV detector (Hitachi, Tokyo, Japan). Data acquisition was performed with LaChrom Elite software (VWR, Darmstadt, Germany). Triplicates of quality control samples at 200, 2,000, and 15,000 ng/ml were used to ensure precision and accuracy during quantification. The lower limit of quantification (LLOQ) was set equal to 24 ng/ml when 250 μl of plasma was used. The LLOQ for plasma samples with sample volumes less than 250 μl was scaled according to the sample volume. In practice, this resulted in an increased LLOQ for samples containing less than 250 μl of plasma.

Pharmacokinetic and statistical analysis. Plasma lumefantrine concentrations were transformed into their natural logarithms, and the concentration-time profiles were modeled by nonlinear mixed-effects population modeling with NONMEM (version VI) software (Icon Development Solutions). The first-order conditional estimation method with interactions was used throughout the modeling (8, 50, 51). The Census program (version 1.0) (58), the S-PLUS program (version 6.0) for Windows (Insightful Corp., Seattle, WA), the pearl-speaks-NONMEM program (PsN; version 2.2.5), and the Xpose program (version 4.0) (21) were used for visual and quantitative diagnostics.

One-, two-, and three-compartment pharmacokinetic models with zero- and first-order absorption with and without an absorption lag time were evaluated. A transit compartment model for the description of absorption was also tried (45). Elimination was assumed to take place from the central compartment. The models were parameterized as clearance (CL/*F*), the apparent volume of distribution in the central compartment (V_C/F), the absorption rate constant (k_a), intercompartmental clearance(s) (Q/F), and the peripheral apparent volume of distribution(s) (V_P/F). *F* is the oral bioavailability. Interindividual random variability in all parameters was modeled exponentially as illustrated for CL: $(CL/F)_i = TV(CL/F) \times \exp(\eta_{i,CL/F})$, where $(CL/F)_i$ is the individually estimated value of CL for the *i*th patient, TV(CL/*F*) is the typical CL value for the modeled population, and $\eta_{i,CL/F}$ is the between-patient random variability assumed to be normally distributed (zero mean, variance ω^2). Correlations between variability components were also evaluated. Additive, proportional, and slope-intercept error models were applied to explain the residual random variability, which originates from intraindividual variability, measurement errors, and model misspecification.

Model discrimination was assessed by a likelihood ratio test by using the objective function values (OFVs) computed by the NONMEM program. The OFV is essentially equal to −2 × log likelihood, and the difference in OFV (ΔOFV) between models is assumed to be χ^2 distributed (7). ΔOFVs of 3.84 and 6.63 are considered to be significant at $P < 0.05$ and $P < 0.01$, respectively, with 1 degree of freedom (i.e., a difference of one parameter) when two competing hierarchical models are compared. The Akaike information criterion (AIC) and the Schwarz information criterion (SIC) were used to compare nonhierarchical models.

All continuous covariates (age, height, weight, body mass index [BMI], estimated gestational age [EGA], initial hematocrit, initial parasitemia, respiratory rate, pulse, body temperature, and biochemical results) and categorical covariates (smoking and primiparity) were investigated by incorporating them separately on each individual parameter. Covariates were also investigated by using the automated function in PsN of stepwise addition (ΔOFV, >3.84, to be included in the model) followed by stepwise deletion (ΔOFV, >6.63, to be retained in the model) of covariates. Different *P* values for the addition and the deletion of covariates were used for a more parsimonious modeling. Categorical and continuous covariates were tried as linear, piecewise linear, and exponential functions centered on the median value.

Diagnostic plots were used to evaluate the overall goodness of fit by plotting the measured log-transformed lumefantrine concentrations versus the population fitted and the individually fitted log-transformed lumefantrine concentrations and by plotting conditional weighted residuals (18) versus time and individually fitted log-transformed lumefantrine concentrations. The extent of individual parameter estimate shrinkage (eta shrinkage), as exemplified for CL, and epsilon shrinkage associated with the perfect-fit phenomenon were calculated by using the following equations: eta shrinkage = $1 - (SD_{\eta CL}/\omega_{CL})$ and epsilon shrinkage = $1 - SD_{IWRES}$, where $SD_{\eta CL}$ is the standard deviation of the individual estimates of η for CL and ω_{CL} is the standard deviation of the estimated population variance. SD_{IWRES} is the standard deviation of the individually weighted residuals. A high shrinkage is the consequence of uninformative data and will result in poor empirical Bayes estimates (eta shrinkage) and a low power to detect model misspecification and residual error misspecification (epsilon shrinkage) (46).

Empirical Bayes estimates of the values of the pharmacokinetic parameters were used to simulate full individual profiles for all patients in the study by using the WinNonLin (version 5) program (Pharsight Corporation). The predicted day 7 capillary lumefantrine concentrations were taken directly from the individually simulated concentration-time profiles. The day 7 venous lumefantrine concentrations were computed from the day 7 capillary concentrations by using the previously published linear correlation in plasma when plasma was sampled on days 3, 4, 5, 6, and 7 after a standard artemether-lumefantrine treatment, i.e., $\ln(\text{capillary plasma}) = 0.52 - 0.95 \times \ln(\text{venous plasma})$ (49). PsN was used to run a nonparametric bootstrap analysis of 2,000 iterations to provide unbiased estimates of the standard errors and the 95% CIs of the estimated parameters. The resampled data sets did not need any stratification, since the samples were evenly distributed over the study period and should accurately represent the structure of the original data set. Visual and numerical predictive checks were used for diagnostics and to characterize the model's simulation properties (17). The final model was used to simulate 1,000 concentrations at each of the individual sampling times for up to 15 days after the initiation of treatment. The population median concentrations and a 90% prediction interval were obtained by extracting the 5th, 50th, and 95th percentiles of their simulated distributions. These were plotted against the observations.

Monte Carlo simulations by use of the final model with observed variability were performed for the different dosing regimens to obtain the population mean concentration-time profiles and 90% prediction intervals after prospective dose adjustments. Forward and backward variable selection logistic regressions were performed with the STATA program (version 10.0; Stata Corp.) to evaluate the potential determinants of the outcome. The risk of a new or a recrudescent infection during gestation was calculated as a proportion by using the STATA program.

TABLE 1. Patient demographics, covariates, and treatment outcome in the clinical study of artemether-lumefantrine in pregnant patients with malaria

Demographic characteristic, covariate, or treatment outcome	Value
Total no. of patients	103
Total dose of lumefantrine (mg/kg)[a]	58.8 (44.3–82.3)
Continuous covariates[a]	
Age (yr)	24 (15–42)
Body wt (kg)	49 (35–65)
Ht (cm)	152 (139–165)
BMI (kg/m^2)	21.1 (17.5–26.7)
Respiratory rate (min^{-1})	24 (16–44)
Pulse (min^{-1})	92 (48–138)
Temp (°C)	36.7 (35–39.3)
Estimated gestational age (wk)	22.6 (13.1–39.0)
Parasitemia (no. of parasites/μl)	4,400 (57–153,734)
Hematocrit (%)	29 (20–42)
Categorical covariates	
Primiparity (%)	22.3
Smokers (%)	44.7
Treatment outcome	
% (no.) of patients with no parasite reappearance	63.1 (65)
% (no.) of patients with new infection[b]	20.4 (21)
Time (days) to new infection[a]	35 (15–140)
% (no.) of patients with recrudescence[b]	16.5 (17)
Time (days) to recrudescence[a]	23 (14–63)

[a] The values are medians (ranges).
[b] As confirmed by PCR.

RESULTS

One hundred three Burmese and Karen pregnant women aged 15 to 42 years with symptomatic uncomplicated *P. falciparum* malaria were enrolled in this population pharmacokinetic study (full demographic characteristics are given in Table 1). The coformulated artemether-lumefantrine treatment was well tolerated, and no serious adverse advents were reported (safety data are reported in full elsewhere [34]).

Pharmacokinetics of lumefantrine. A total of 585 lumefantrine capillary plasma samples were analyzed. Fifteen of these samples displayed concentrations below the LLOQ. The coefficients of variation (CV) during the quantification of lumefantrine were 5.1%, 2.6%, and 1.9% at 200 ng/ml, 2,000 ng/ml, and 15,000 ng/ml, respectively. Measured concentrations below the LLOQ (<2.6% of total samples) were coded as missing data (6).

Plasma lumefantrine concentration-time data were best described by an open two-compartment disposition model with first-order absorption and lag time. One-compartment models showed systematic model misspecifications in diagnostic plots. A two-compartment structural model did not display any trends in diagnostic plots and resulted in major decreases in AIC and SIC compared to the values achieved with a one-compartment model (change in AIC [ΔAIC] = −283, change in SIC [ΔSIC] = −275). A three-compartment model showed increases in AIC and SIC (ΔAIC = +0.8, ΔSIC = +9.5) and provided no advantage over a two-compartment model in terms of the improvement in diagnostic plots. Furthermore, a three-compartment model resulted in a very poor accuracy in additional key parameters, implying that this model was overparameterized. Zero-order absorption, transit compartment absorption, and first-order absorption without a lag time were inferior to the final absorption model in terms of the diagnostic plots and/or the change in OFV/SIC. A full correlation matrix between variability components (CL/*F*, V_C/F, Q/F, and V_P/F) showed significant improvements in OFVs, SIC, and diagnostic plots. An additive residual error model was adequate to describe the random residual variability, as expected when log-transformed heteroscedastic data are modeled. No additional benefit in terms of the ΔOFV could be seen by adding a proportional residual error term. The selected two-compartment model with first-order absorption and an additive residual error model were used as the base models for evaluation of the covariates. Interindividual variability in all parameters except lag time with a correlation between the variability components mentioned above was also incorporated into the base model.

The following parameters and covariates showed a significant and linear correlation when the covariates were added separately to individual parameters in the base model: k_a-EGA (ΔOFV = 11.9) V_C/F-EGA (ΔOFV = 10.4), CL/*F*-smoking (ΔOFV = 8.7), V_P/F-respiratory rate (ΔOFV = 6.9), CL/*F*-

pulse (ΔOFV = 5.7), CL/*F*-respiratory rate (ΔOFV = 4.1), V_P/F-body weight (ΔOFV = 4.1), and CL/*F*-age (ΔOFV = 3.9). EGA as a covariate on V_C/F instead of on k_a was selected as the starting point for the forward addition approach because of the similar OFVs and a more mechanistically plausible model. Interindividual variability and the accuracies of all key parameters were also comparable between the two models. Most of these covariates showed a significant improvement when they were added in a stepwise manner, but only EGA on V_C/F and smoking on CL/*F* could be retained in the backward elimination step with a higher significance level. A model that included both smoking and EGA as covariates showed unacceptably poor precision in parameter estimates (e.g., >230% CV for V_C/F and >50% CV for CL/*F*) and no major improvement on interindividual variability (51.3% CV versus 52.2% CV interindividual variability [IIV] in CL/*F*) or in diagnostic plots. The final population pharmacokinetic model incorporated only EGA on V_C/F as a linear covariate centered on the median value. The final parameter estimates and variability components are described in Table 2.

Basic goodness-of-fit plots are shown in Fig. 1. No substantial eta shrinkage was computed for the key parameters in the final model: CL/*F* = 1.79%, V_C/F = 16.2%, Q/F = 5.86%, V_P/F = 4.96%, and k_a = 16.5%. Epsilon shrinkage was computed to be 16.6%. A visual predictive check was used for both the random and the fixed effects and showed that the expected clinical variability was representatively described in the final model (Fig. 2). Typically, one would expect the central tendencies of simulated data to match the observations, and approximately 10% of the observations should be distributed equally above and below the 90% prediction limits. A numerical predictive check showed that 10.3% of the observed values were outside the 90% prediction interval, as expected.

Pharmacodynamics of lumefantrine. Twenty-one patients presented with a new infection at a median of 35 days (range, 15 to 140 days) of follow-up. Recrudescent infections occurred in 17 patients at 23 days (range, 14 to 63 days) of follow-up. Therefore, the incidences of PCR-confirmed new infections and recrudescent infections until delivery were 20.4% (95% CI, 13.1 to 29.5) and 16.5% (95% CI, 9.9 to 25.1), respectively. There was a nonsignificant (P = 0.26) trend for the predicted day 7 median capillary lumefantrine concentrations to be lower in women in whom *P. falciparum* reappeared, that is, 388 ng/ml (range, 126 to 546 ng/ml) in women who went on to have recrudescent infections (n = 17) and 377 ng/ml (range, 136 to 1,210 ng/ml) in women who contracted new infections (n = 21) but 427 ng/ml (range, 135 to 1,600 ng/ml) in women in whom the parasite did not reappear (n = 65). A high proportion of all women (40%, 41/103) had day 7 capillary plasma concentrations of <355 ng/ml (i.e., a concentration that tentatively corresponded to <280 ng/ml in venous plasma), a threshold previously associated with increased rates of failure in nonpregnant patients (11, 12, 49). The trend could also be seen in the cumulative risk for recrudescence with both exposure (AUC from 72 to 400 h) and the day 7 plasma concentrations of lumefantrine (Fig. 3). No treatment failures occurred in patients with day 7 capillary plasma lumefantrine concentrations above 550 ng/ml, which would correspond to 360 ng/ml in venous plasma.

Potential predictors of recrudescence were also investigated by the use of logistic regression. Only EGA was statistically significant as a predictor of recrudescence by using forward variable selection (Table 3). The model indicated a 12% odds increase in recrudescence for each successive week of EGA on admission for the pregnant women in this study. The predicted day 7 plasma lumefantrine concentrations, smoking, type of infection at admission (novel or recrudescent), BMI, and EGA were all significant predictors by use of the backward variable selection approach (Table 3). High predicted day 7 lumefantrine concentrations, smoking, and the presence of novel infections rather than recrudescent infections at admission were associated with a reduced risk of treatment failure in this group. Patients with a high EGA and BMI at admission showed an increased risk of recrudescence.

Dose regimen simulations. As the responses to treatment were poor and the lumefantrine concentrations were low, dif-

TABLE 2. Parameter estimates of the final two-compartment model describing lumefantrine population pharmacokinetics in pregnant patients with malaria

Parameter[a]	Population estimate[b]	95% CI[c]	% RSE[c]
CL/*F* (liters/h)	6.11	5.46–6.76	5.38
V_C/F (liters)	20.2	10.47–36.03	33.4
Q/F (liters/h)	1.82	1.5–2.17	9.14
V_P/F (liters)	160	134.68–193	9.32
k_a (h^{-1})	0.0588	0.052–0.068	6.90
t_{lag} (h)	1.67	0.37–2.29	30.2
$\theta_{V_C/F\text{-}EGA}$	0.0721	0.031–0.101	23.3
σ (% CV)	10.6	7.56–13.5	28.8
Interindividual variability (% CV)			
$\eta_{CL/F}$	0.273 (52.2)	0.202–0.357	15.3
$\eta_{V_C/F}$	2.05 (143.2)	1.21–3.377	29.6
$\eta_{Q/F}$	0.595 (77.1)	0.39–0.864	22.0
$\eta_{V_P/F}$	0.619 (78.7)	0.334–1.011	33.4
η_{ka}	0.135 (36.7)	0.0608–0.211	28.4
Covariance (correlation)[d]			
$\eta_{CL/F} \sim \eta_{V_C/F}$	0.480 (0.64)	0.257–0.745	26.9
$\eta_{CL/F} \sim \eta_{Q/F}$	0.344 (0.85)	0.232–0.476	19.3
$\eta_{CL/F} \sim \eta_{V_P/F}$	0.353 (0.86)	0.231–0.510	22.5
$\eta_{V_C/F} \sim \eta_{Q/F}$	0.493 (0.45)	0.183–0.847	39.0
$\eta_{V_C/F} \sim \eta_{V_P/F}$	0.779 (0.69)	0.388–1.293	33.5
$\eta_{Q/F} \sim \eta_{V_P/F}$	0.532 (0.88)	0.328–0.807	27.1
Secondary parameters [median value (range)]			
V_{SS}/F (liters)[e]	181 (29–1,493)		
$t_{1/2}$ (days)[e]	3.3 (1.5–7.8)		
$AUC_{0-\infty}$ (mg × h/liter)[e]	472 (119–1,261)		
Day 7 lumefantrine concn (ng/ml)[e]	391 (126–1,600)		

[a] t_{lag}, absorption lag time; $\theta_{V_C/F\text{-}EGA}$, fractional change in V_C/F per week change in EGA from the median value; σ, additive residual error; η, interindividual variability; V_{SS}, steady-state volume of distribution; $t_{1/2}$, terminal elimination half-life; RSE, relative standard error. The other abbreviations are defined in the text.

[b] Typical value assessed by use of the NONMEM program.

[c] Assessed by nonparametric bootstrap method with 2,000 iterations. Runs without a successful conclusion (minimization successful) were omitted from the analysis (n = 894); 1,106 runs in total are included.

[d] Correlations are calculated as $\eta_1 \sim \eta_3 = \eta_2/\sqrt{(\eta_1 \times \eta_3)}$.

[e] Model-based estimates of individual values were calculated from empirical Bayes estimates of parameters.

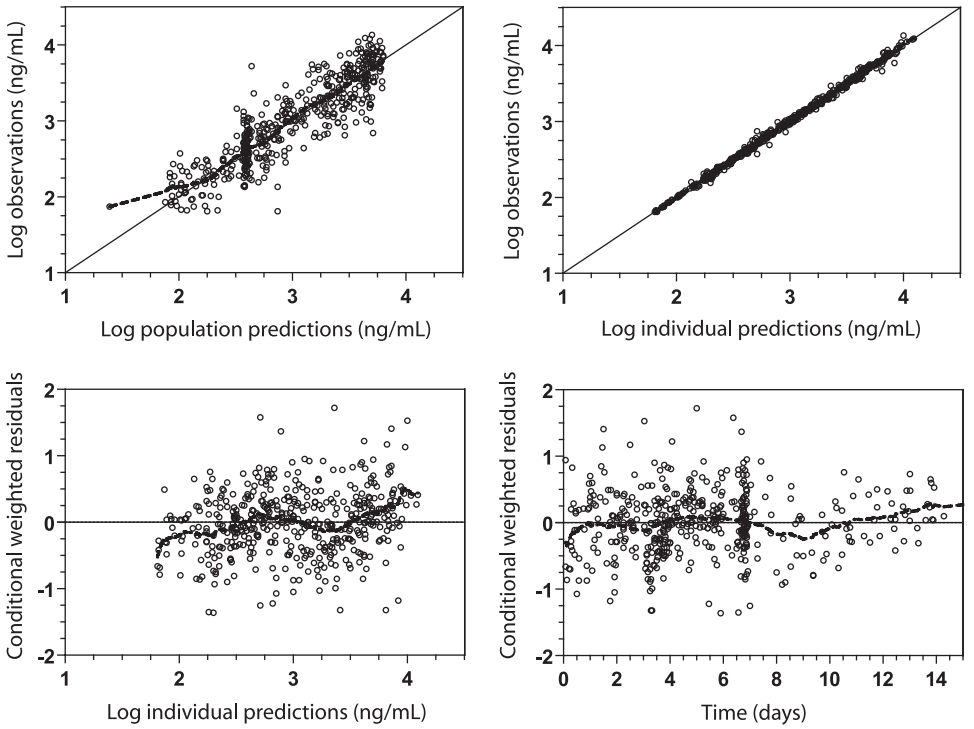

FIG. 1. Goodness of fit for the final two-compartment model describing the population pharmacokinetics of lumefantrine in pregnant patients with uncomplicated *P. falciparum* malaria. Broken line, a locally weighted least-squares regression; solid line, line of identity. The observed capillary plasma lumefantrine concentrations, population predictions, and individual predictions were transformed into their logarithms (base 10).

ferent dose regimens were simulated at the population level by using the final model with the observed variability and the PsN and NONMEM programs. Dose-linear pharmacokinetics were assumed in the simulated concentration-time profiles, even though previous studies have shown that lumefantrine is absorbed in a dose-dependent manner in nonpregnant adults (5, 12). Dose escalation was performed for the standard treatment

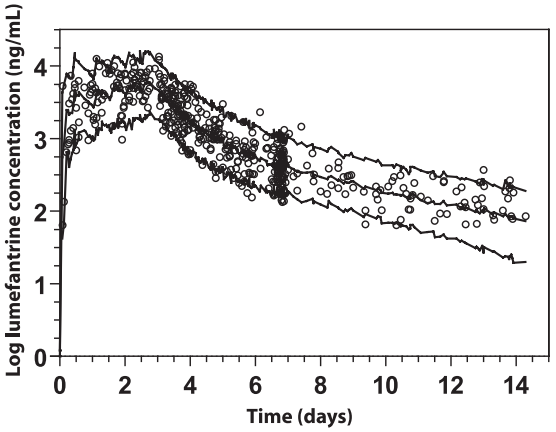

FIG. 2. Visual predictive check of the final two-compartment model describing the population pharmacokinetics of lumefantrine in pregnant patients with uncomplicated *P. falciparum* malaria. Open circles, observed data points; solid lines, representation of the simulated (n = 1,000) 5th, 50th, and 95th percentiles of the data. Capillary plasma lumefantrine concentrations were transformed into their logarithms (base 10).

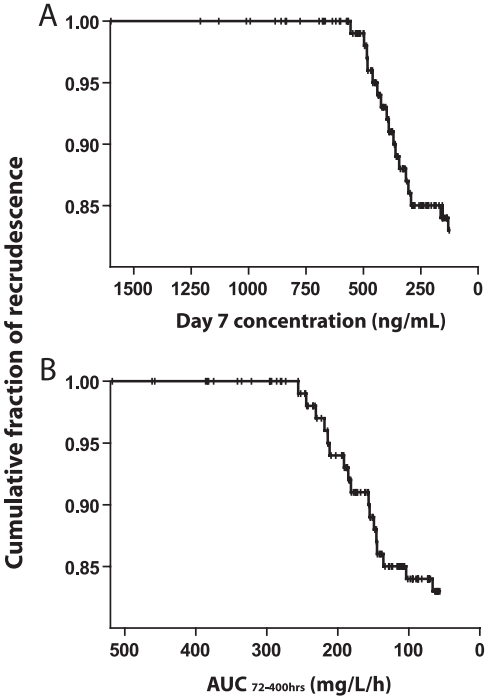

FIG. 3. Cumulative fraction of recrudescence versus day 7 capillary plasma lumefantrine concentrations (A) and lumefantrine drug exposure (AUC) from 72 h to 400 h after drug initiation (B). Vertical lines, predicted concentration or exposure (n = 103).

TABLE 3. Predictors of recrudescence in pregnant patients with uncomplicated *P. falciparum* malaria using logistic regression[a]

Selection method and variable	Odds ratio[b]	z[c]	$P > \|z\|$[d]	95% CI[e]
Forward variable selection				
EGA (wk)	1.12	2.71	0.007	1.03–1.22
Backward variable selection				
EGA (wk)	1.17	2.63	0.008	1.04–1.31
Body mass index (kg/m^2)	1.64	2.39	0.017	1.09–2.45
Novel infection at admission	0.16	−2.45	0.014	0.038–0.70
Smoking	0.13	−2.47	0.014	0.026–0.66
Predicted day 7 lumefantrine concn (ng/ml)	0.995	−2.36	0.018	0.99–1.0

[a] Data are for 98 patients. Information on the type of infection at admission was missing for five patients.
[b] The odds ratio gives the expected relative change in odds when there is a 1-unit change in the value of the predictor variable when the values of all of the other variables in the model are held constant.
[c] The z statistic was used to test the hypothesis that the logistic regression coefficient is equal to 0.
[d] Two-tailed P value for the z test.
[e] The 95% CIs for the logistic regression coefficients.

duration of 3 days until the 5th percentile of the capillary lumefantrine concentrations reached the target level of 355 ng/ml on day 7. A 100% dose increase (eight tablets per dose) was needed to achieve the target concentration. In contrast, higher concentrations were achieved on day 7 with a 50% dose increase (six tablets per dose) when the six doses were given over 5 days instead of over 3 days. A small increase in the concentrations on day 7 could be seen when artemether-lumefantrine was given three times a day for 3 days compared with the concentrations achieved with the normal standard-dose regimen. An increased duration of artemether-lumefantrine treatment was necessary for a major impact on the concentrations on day 7 to be achieved without a major dose increase. The results of population simulations of three different dose regimens are shown in Fig. 4. No simulations of higher-dose regimens are shown because of the dose-dependent oral absorption of lumefantrine (5, 12). The duration of artemether and dihydroartemisinin exposure is assumed to last for approximately 6 h after each dose (i.e., three half-lives). This is shown in Fig. 4 in order to illustrate the timing and duration of the different dose regimens. A 5-day regimen would provide artemether and dihydroartemisinin exposures in three consecutive parasite cycles (compared with two for the current 3-day regimens) and a major increase in the day 7 plasma lumefantrine concentrations. Both these factors should ensure maximum cure rates.

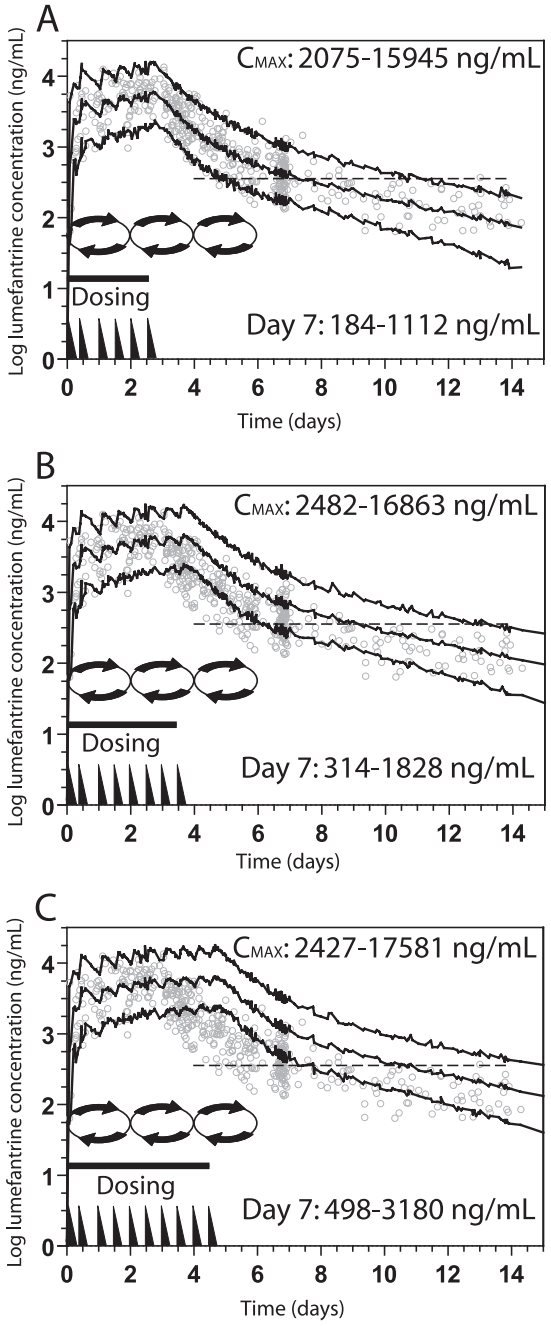

FIG. 4. Visual predictive checks of the final two-compartment model describing the population pharmacokinetics of lumefantrine in pregnant patients with uncomplicated *P. falciparum* malaria for different dose regimens. (A) Standard treatment of four tablets of artemether-lumefantrine (one tablet contains 20/120 mg artemether/lumefantrine) twice a day for 3 days (0, 8, 24, 36, 48, and 60 h); (B) four tablets twice a day for 4 days (0, 8, 24, 36, 48, 60, 72, and 84 h); (C) four tablets twice a day for 5 days (0, 8, 24, 36, 48, 60, 72, 84, 96, and 108 h). Open gray circles, observed data points from the study; solid black lines, representation of the simulated (n = 1,000) 5th, 50th, and 95th percentiles for each dose regimen; broken black line, target of 355 ng/ml in day 7 capillary plasma lumefantrine concentrations; one circular black arrow, one parasite life cycle of 48 h; bold horizontal black blocks, duration of dosing; black solid triangles, an approximation of the duration of artemether and dihydroartemisinin exposure (three half-lives, or ≈6 h) during the simulated dose regimen. Capillary plasma lumefantrine concentrations were transformed into their logarithms (base 10).

DISCUSSION

The fixed-dose oral antimalarial combination treatment artemether-lumefantrine is the most widely used ACT in the world today and is recommended as the first-line treatment in many tropical countries. ACTs are now considered the treatment of choice for uncomplicated malaria during the second and third trimesters in pregnancy (57). It is essential that the safety and pharmacokinetics of this treatment be well characterized for this vulnerable group. There are still limited pharmacokinetic data on lumefantrine, although an increasing

number of clinical studies have reported day 7 plasma lumefantrine concentrations. Two population studies of the pharmacokinetics of lumefantrine in nonpregnant patients have been reported over the last decade (11, 12), and we have also reported on a small conventional pharmacokinetic clinical study with 13 pregnant women who were frequently sampled (32). The present study is a much larger investigation of the population pharmacokinetics of lumefantrine in this vulnerable group of patients receiving the fixed oral combination of artemether-lumefantrine. The plasma concentration data obtained in this study cannot be compared directly with published results for nonpregnant individuals since different matrices were used for drug measurement (capillary versus venous plasma).

Population pharmacokinetics were generally well described by the final two-compartment model with first-order absorption and elimination. Multicompartment pharmacokinetics have previously been described for lumefantrine, and the present model has a mechanistically plausible structure and describes the data accurately. No major trends could be seen in goodness-of-fit plots or simulation-based diagnostics, which also suggests that there is no major model misspecification. A small trend of the under- and overprediction of high and low lumefantrine concentrations, respectively, can be seen in Fig. 1. However, the use of a three-compartment model could not be supported by these data. The absorption lag time for lumefantrine has not been described before and could be related to the delayed gastric emptying that occurs in late pregnancy. Other absorption models, for example, a more mechanistic transit compartment absorption model, did not improve the fit. This might be because the data in the absorption part of the curve were too sparse, in combination with a very slow absorption and multiple frequent dosing. As in previous studies, the plasma lumefantrine concentration data showed large interindividual variability in all pharmacokinetic parameters, which was predominantly translated into poor precision on the absorption lag time and V_C/F. The inclusion of EGA as a covariate decreased the interindividual variability in V_C/F but could not fully explain the large observed variability which presumably results from the variable absorption (F) of the lipophilic compound lumefantrine. Other parameters were well characterized with satisfactory accuracy.

A 7.2% increase in the V_C/F per EGA (week) from the median EGA confirms that the pharmacokinetics of lumefantrine are altered during the time course of pregnancy. This might also imply that the pharmacokinetics in pregnant women in late pregnancy are different from those in nonpregnant women, as suggested in our earlier report. Circulating lumefantrine is highly bound to high-density lipoproteins (HDLs), but HDL levels are increased during pregnancy (16), which would result in a decrease in the volume of distribution and raise the total plasma lumefantrine concentrations. The observed changes can therefore not be attributed to the altered plasma protein binding of lumefantrine to HDLs. Altered tissue distribution is one possible explanation, but that would preferentially affect the peripheral volume of distribution. EGA was shown not to be a significant covariate on V_P/F, and the increase in V_C/F is therefore likely to result from the physical alterations resulting from the increased plasma volume in pregnant women. The possibility of alterations in other lumefantrine pharmacokinetic parameters (e.g., CL/F) cannot be excluded, but the data presented here do not support this in terms of pregnancy-related covariates. Gender has previously been shown in larger series ($n = 266$) to not modify the pharmacokinetics of lumefantrine in Thailand (12). Thus, gender per se should not explain the differences between nonpregnant adults and pregnant women.

The observed median day 7 capillary plasma lumefantrine concentration of 433 ng/ml (range, 134 to 1,454 ng/ml) was in good agreement with the individually predicted day 7 concentrations from profiles simulated for the same 85 patients (median, 411 ng/ml; range, 135 to 1,600 ng/ml). The predicted day 7 capillary lumefantrine concentrations (median, 391 ng/ml; range, 126 to 1,600 ng/ml) were therefore used for all 103 pregnant women enrolled in this study to utilize all possible data. This approach also avoids the potential bias from the censuring of data. These predicted day 7 capillary plasma concentrations were approximately equivalent to the median venous plasma concentrations of 310 ng/ml (range, 94 to 1,364 ng/ml) determined by using the previously characterized relationship between capillary and venous concentrations (49). This is considerably lower than the corresponding values previously reported in nonpregnant adults and pediatric patients with multiresistant malaria in Thailand, Cambodia, and the Lao People's Democratic Republic (10, 29, 42, 47). Price et al. (42) reported a median day 7 venous plasma lumefantrine concentration of 528 ng/ml (range, 49 to 5,175 ng/ml) in Karen patients ($n = 201$) receiving the same standard treatment of artemether-lumefantrine. Nonpregnant adult patients ($n = 77$) from the Lao People's Democratic Republic had a mean venous day 7 plasma lumefantrine concentration of 470 ng/ml (95% CI, 380 to 560 ng/ml) (29). The mean day 7 venous plasma lumefantrine concentration in the group ($n = 64$) with an adequate therapeutic response in Cambodia was 860 ng/ml, and the mean concentration in the group ($n = 10$) with late treatment failure was 510 ng/ml (10). A median day 7 venous plasma lumefantrine concentration of 932 ng/ml (range, 127 to 3,094 ng/ml) was observed in the Karen population ($n = 75$) when they received the same total dose over 5 days (0, 8, 24, 48, 72, and 96 h) instead of 3 days (0, 8, 24, 36, 48, and 60 h) (42). The incidence of failure was also significantly lower among the nonpregnant adults and children than among the same population of pregnant women in the present study: 3.2% (95% CI, 1.8% to 4.6%) and 16.5% (95% CI, 9.9% to 25.1%), respectively (42). The unacceptably high proportion of treatment failures in the present study is therefore likely to result from altered pharmacokinetics. It is also worrying that 53% (9/17) of these failures occurred within 3 weeks after treatment, with 2 cases occurring as early as 2 weeks after enrollment.

The logistic regression model indicated a 12% increase in the odds of recrudescence for each week of increase in EGA at admission, which confirms that women enrolled at a late stage of pregnancy are at a higher risk of recrudescence than women enrolled at an early stage of pregnancy. EGA is also a significant covariate on V_C/F in the pharmacokinetic model. This implies that the risk of treatment failure and that the pharmacokinetics of lumefantrine in pregnant women change over the time course of the pregnancy. Backward variable selection computed a 64% higher odds for recrudescence with each 100-ng/ml decrease in the day 7 lumefantrine concentrations.

Thus, high day 7 lumefantrine concentrations are associated with a reduced risk of treatment failure, which supports the suitability of the use of the concentrations on day 7 as a surrogate marker for treatment failure or success. However, this is a fixed combination treatment in which both artemether and lumefantrine have parasiticidal effects. Total drug exposure and the maximal concentrations of artemether and dihydroartemisinin are also reported to be lower in pregnant women than in nonpregnant adults (32). This might explain why the day 7 lumefantrine concentrations were not selected in the forward variable selection for pharmacokinetic-pharmacodynamic relationships. Smoking was shown to be protective for recrudescence, which may be a random effect due to the small numbers. It was not possible to explore this further by use of this small data set. This needs to be investigated thoroughly with a larger series. Dihydroartemisinin is eliminated by glucuronidation, most likely mediated by UGT1A9 and UGT2B7 (19). UGT2B7 exhibits only low nicotine glucuronidation activity and is essentially inactive toward cotinine. UGT1A9 does not glucuronidate nicotine or cotinine (22). Artemether is metabolized by the cytochrome P450 (CYP450) enzymes CYP1A2, CYP2B6, CYP2C19, and CYP3A4 in vitro (38); but no contribution by CYP2C19 could be seen in healthy volunteers (48). Lumefantrine is mainly metabolized by CYP3A4 (26–28). In vitro studies have shown that CYP2A6 is the most important CYP450 enzyme in the metabolism of nicotine (36, 37). It is therefore unlikely that the protective effect of smoking would result from inhibition of the enzyme systems involved in the metabolism of artemether, dihydroartemisinin, and/or lumefantrine by nicotine. Not surprisingly, novel infections at admission were associated with a reduced risk of recrudescence compared to the risk when treating recrudescent infections at admission. Less sensitive parasites are expected to be selected in a recrudescent infection and are therefore harder to treat, resulting in less favorable outcomes. A high BMI was also associated with an increased risk of recrudescence, and this might reflect an underlying correlation between BMI and pharmacokinetics. A high BMI might be correlated with a large volume of distribution and/or a high clearance, which would give a lower level of total drug exposure and therefore an increased risk of recrudescence.

Evidently, pregnant women in this area are not getting the right artemether-lumefantrine dose regimen. The median day 7 concentrations are approximately 60% of those in nonpregnant adults and children in the same area (42). Similarly, the present study reports that a high percentage of patients (i.e., 40%) had concentrations below the previously described median day 7 concentration cutoff for determination of the risk of therapeutic failure (12). The lower levels of exposure to artemether, dihydroartemisinin, and lumefantrine result in unacceptably high failure rates; and a dose adjustment is urgently needed for these women so that an adequate treatment response can be achieved. A number of different dose regimens were simulated at the population level to evaluate potential improved treatment regimens. The simulations suggested that a 100% dose increase would achieve the target day 7 plasma lumefantrine concentrations in 95% of these women with a normal 3-day regimen. This may not achieve its objective because of the dose-limited absorption of lumefantrine (5). Therefore, we suggest the use of a 5-day instead of a 3-day course of artemether-lumefantrine in later pregnancy. This is also supported by previous studies showing higher cure rates when the same total dose is given over 5 days (0, 8, 24, 48, 72, and 96 h) instead of the normal 3 days (12, 42). However, shorter treatment regimens are likely to increase compliance. Hence, a compromise such as a 4-day treatment (0, 8, 24, 36, 48, 60, 72, and 84 h) with an increased dose could prove to be a viable alternative. The initial response and parasite clearance times were mainly attributed to the artemisinin component of the combination, and these were similar for the different treatment regimens (12). However, a recent study suggests that the parasite clearance times in western Cambodia are prolonged due to decreased susceptibility to artemisinin (39). Therefore, it might not be wise to decrease the number of artemether doses in each parasite cycle since it might select for resistant parasite strains. The total exposure to artemether and dihydroartemisinin were previously shown to be approximately 50% and 40% lower in pregnant women, respectively, than in adult nonpregnant patients (32). No dose-dependent absorption has been reported for artemether, and a dose increase to achieve concentrations of artemether and dihydroartemisinin similar to those in nonpregnant patients is appropriate. A dose duration covering three parasite cycles (>96 h) is theoretically preferable, although it may be associated with reduced adherence and increased cost. The safety aspects of the use of a prolonged treatment and/or increased doses also need to be evaluated. No major effect of a prolonged treatment on maximum lumefantrine concentrations could be seen, which suggests that no increase in acute toxicity would occur on account of an increased duration of treatment with lumefantrine (Fig. 4).

In conclusion, this is the first population pharmacokinetic study of lumefantrine in pregnant women with uncomplicated *P. falciparum* malaria. The pharmacokinetics of lumefantrine in this population were well described by a two-compartment model with a linear relationship between V_C/F and EGA. The pregnant women in this study had unacceptably high failure rates due to altered pharmacokinetics. Population-based simulations suggest an increased dose and treatment duration are needed for adequate drug exposure in these patients (e.g., a regimen of treatment twice daily for 5 days). Further safety, efficacy, and pharmacokinetic studies are urgently needed to evaluate the effects of these increased doses in this vulnerable population.

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